

(FILE 'HOME' ENTERED AT 17:59:15 ON 09 MAR 2004)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 17:59:54
ON 09 MAR 2004

L1 86295 S CELL? (6P) (DETERGENT? OR SURFACTANT? OR LYTIC OR ?LYSIS OR ?
L2 41794 S CELL? (6P) (DETERGENT? OR SURFACTANT?) (6P) (LYTIC OR ?LYSIS
L3 1035 S CELL? (6P) (NEUTRAL? OR SEQUEST?) (6P) (CYCLODEXTRIN)
L4 9009 S L1 (6P) L2
L5 548 S L1 (6P) L3
L6 90 S L4 (6P) L5
L7 90 DUP REM L6 (0 DUPLICATES REMOVED)
L8 69988 S (CELL OR CELLS OR CELLULAR) (6P) (DETERGENT? OR SURFACTANT? O
L9 35398 S (CELL OR CELLS OR CELLULAR) (6P) (DETERGENT? OR SURFACTANT?)
L10 619 S (CELL OR CELLS OR CELLULAR) (6P) (NEUTRAL? OR SEQUEST?) (6P)
L11 6954 S L8 (6P) L9
L12 333 S L8 (6P) L10
L13 66 S L12 (6P) L11
L14 66 DUP REM L13 (0 DUPLICATES REMOVED)
L15 29 S L14 (6P) INTRACELLULAR?

L15 ANSWER 27 OF 29 USPATFULL on STN

ACCESSION NUMBER: 2001:78884 USPATFULL
TITLE: Method for analyzing intracellular components
INVENTOR(S): Hattori, Noriaki, Chiba, Japan
Yajitate, Keiko, Chiba, Japan
Nakajima, Motoo, Chiba, Japan
Murakami, Seiji, Chiba, Japan
PATENT ASSIGNEE(S): Kikkoman Corporation, Chiba, Japan (non-U.S.
corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6238857	B1	20010529
	WO 9928495		19990610
APPLICATION INFO.:	US 2000-555682		20000602 (9)
	WO 1998-JP5407		19981201
			20000602 PCT 371 date
			20000602 PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1997-347336	19971203
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Horlick, Kenneth R.	
ASSISTANT EXAMINER:	Strzelecka, Teresa	
LEGAL REPRESENTATIVE:	Foley & Lardner	
NUMBER OF CLAIMS:	10	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	7 Drawing Figure(s); 4 Drawing Page(s)	
LINE COUNT:	769	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for analyzing an intracellular component comprising the following steps, and a reagent kit comprising (a) an extraction reagent, (b) branched dextrin or a derivative thereof, and (c) a reagent for analyzing an intracellular component:

- (1) step of adding an extraction reagent to a sample containing cells to extract the intracellular component;
- (2) step of adding branched dextrin or a derivative thereof to the sample containing the extraction reagent; and
- (3) step of analyzing the extracted intracellular component.

L15 ANSWER 28 OF 29 USPATFULL on STN

ACCESSION NUMBER: 1998:1634 USPATFULL
TITLE: Methods and kits for preparing nucleic acids using cyclodextrin
INVENTOR(S): Lundin, Arne, Dalaro, Sweden
Anson, John George, Cardiff, Wales
Kenrick, Michael Kenneth, Cardiff, Wales
PATENT ASSIGNEE(S): Amersham International plc, Buckinghamshire, United Kingdom (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5705345		19980106
APPLICATION INFO.:	US 1996-645688		19960514 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1994-347228, filed on 23 Nov 1994, now patented, Pat. No. US 5558986 which is a continuation of Ser. No. US 1992-75484, filed on 10 Jan 1992, now patented, Pat. No. US 9200056		

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1991-551	19910110
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Horlick, Kenneth R.	
LEGAL REPRESENTATIVE:	Wenderoth, Lind & Ponack	
NUMBER OF CLAIMS:	17	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	35 Drawing Figure(s); 22 Drawing Page(s)	
LINE COUNT:	1247	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of preparing nucleic acids by obtaining an impure nucleic acid preparation, treating said preparation with phenol and adding a cyclodextrin to the treated preparation to neutralize the phenol.

SUMM The present invention relates to a method for extraction of **intracellular** components including **intracellular** metabolites. The invention addresses the problem that many substances used for extracting components from **cells** interfere with assays or other processing steps performed on the extracted components. The invention uses cyclodextrins to **neutralise** the extracting substances. In one example according to the invention, the **intracellular** metabolite is adenosine triphosphate (ATP) which can, after **neutralisation** of the extractants, be assayed using a firefly luciferin-luciferase reaction. In another example, the **intracellular** components are nucleic acids which can, after **neutralisation** of the extractants, be amplified or further processed in other ways.

SUMM General aspects of extraction of **intracellular** components

SUMM The assay of **intracellular** components in biological samples is often performed by enzymatic methods. Such methods require: 1) Release of the components from the **cells** to make the components available to enzyme systems added in the assay. 2) Inactivation of enzymes from the **cells** that may act on the components during preparation, storage or assay of extracts. Extraction of the **intracellular** components involves opening of **cell** walls and membranes and release of the entire metabolite pools into the surrounding medium. Within the **cells** the metabolite pools often have turn-over times around a few seconds due to the action of the **intracellular** enzymes. As soon as an extractant starts to affect membrane integrity the enzyme systems of the **cell** try to counteract the resulting effects. Thus considerable changes of metabolite levels may take place during an extraction which takes time. This would obviously result in completely erroneous data on

intracellular metabolite levels even using the best enzymatic assays. The only way to avoid the problem is to use extractants that rapidly open up the **cell** membranes and simultaneously inactivate all enzymes that act on the **intracellular** components. Enzyme inactivation is therefore an inherent property of all reliable extractants. The presence of a **cell** wall protects the **cell** from the extractant and makes bacterial, fungal and algal **cells** particularly difficult to extract. Thus strong acids with chaotropic anions like trichloroacetic acid (TCA) or perchloric acid (PCA) have frequently been used for the extraction of these types of **cells**. Such agents are strongly enzyme inactivating and inevitably interfere with enzymatic assays unless extracts are highly diluted before the assay.. . .

SUMM The more rapid the turn-over rate of the intermediate metabolite the higher is the requirement for immediate inactivation of **cellular** enzymes at the addition of the extractant. From this point of view ATP is one of the most difficult **intracellular** metabolites to extract. In all **cells** ATP is the means by which energy is transferred from energy yielding to energy requiring reactions. Thus many ATP converting. . . have high activities. Even a slight damage of membrane integrity, e.g. by an extractant, results in a rapid loss of **intracellular** metabolites and ions. As the **cell** tries to compensate for these events large quantities of ATP are consumed. One object of the work leading to this. . . method for microbial ATP compatible with the firefly luciferase assay. The rapid turn-over of ATP and the presence of thick **cell** walls in microbial **cells** make it likely that an extraction method for microbial ATP will work also for most other **intracellular** metabolites in any type of **cell** (unless the extractant by itself degrades the metabolite). Furthermore in the firefly luciferase assay of ATP the rate of the. . .

SUMM . . . is required as a substrate or template for subsequent enzymatic reactions, and hence must be biologically active. Commonly, DNA from **cells** or tissue is used for the amplification of specific sequences by the polymerase chain reaction (PCR) or cleavage with restriction enzymes for gene cloning or identification. The purification of genomic DNA from **cells** or tissue for subsequent use in gene **analysis** experiments conventionally involves **cell** **lysis** to release all **cellular** components, followed by selective digestion of proteins and RNA with specific degradative enzymes. After separation from proteinaceous material and other. . . where functionally active genomic DNA can be prepared without specific removal of contaminating protein, for example by ethanol precipitation of **cell** lysates (H. Xu, A. M. Jevnikar and E. Rubin-Kelly, Nucleic Acids Research 18, 4943). The critical contaminant therefore appears to be the extractant used, which is conventionally a **detergent**. Removal of the **detergent** can therefore be sufficient to allow the DNA to be used for subsequent reactions. However, conventionally **detergent** removal still requires a separation step, with the subsequent increase in preparation time and potential reduction in yield. A homogeneous. . .

SUMM In rapid microbiology the firefly luciferase assay of ATP is frequently used for biomass estimations. The **intracellular** ATP concentration is similar in all **cells** and the amount of ATP per **cell** is approximately proportional to the **intracellular** volume. Bacteria contain approx. 10^{-18} moles of ATP per **cell** while fungi and algae contain considerably more ATP per **cell**. With simple light measuring instruments and firefly luciferase reagents 10^{-15} moles of ATP is easily detected in a 1 ml volume. This corresponds to approx. 10^{-3} bacterial **cells**. Bacterial ATP in a biological specimen can be extracted by adding an equal volume of 2.5% trichloroacetic acid. However, to. . . be used in a final assay volume of 1 ml. Thus the detection limit in the biological specimen is 10^{-5} **cells/ml**.

Neutralisation of the acid improves the situation somewhat but most of the inhibition comes from the chaotropic anion of the acid.

SUMM . . . Press, New York, 1984). The important finding was, however, that the luciferase inactivating effect of quaternary ammonium compounds could be **neutralised** although albumin was not ideal for the purpose. An alternative **neutralising** agent for quaternary ammonium compounds was later found to be nonionic **surfactants**, e.g. Tween 20, Tween 60, Tween 80, Polyoxyethylene ether W1 and Triton X-100 (W. J. Simpson and J. R. M. Hammond, EP 309184). S. Kolehmainen and V. Tarkkanen have proposed (GB 16004249) the use of nonionic **surfactants** as extractants in their own right. Nonionic **surfactants** counteract the gradual inactivation of luciferase by quaternary ammonium compounds and are not by themselves strongly inhibitory in the luciferase. . . inhibition of the luciferase reaction is obtained at the addition of quaternary ammonium compounds even in the presence of nonionic **surfactants** (cf. Example 1). Thus no system has been described that obviates both problems with quaternary ammonium compounds, i.e. inhibition and. . .

SUMM . . . market for such assays is actually field testing under non-laboratory conditions using personnel with little or no training in biochemical **analysis**. Under such conditions assays would normally involve low numbers of samples in each series and would have to be performed. . .

SUMM Very potent extractants that rapidly penetrate the **cell** wall and inactivate the **intracellular** enzymes have to be used with microbial **cells**. The interference with enzymatic **analysis** from such extractants can be obviated by: 1) Dilution of extracts (resulting in a reduced sensitivity of the assay). 2) Removal of the extractant from the extract (most likely resulting in time-consuming and laborious procedures). 3) **Neutralisation** of the extractant by including a **neutralising** agent in the assay buffer. The last suggestion is obviously the most attractive alternative. The requirement for very potent extractants also makes it difficult to achieve. The situation is not simplified by the fact that the **neutraliser** has to be relatively inert with no effects on luciferase activity.

SUMM . . . aim of this aspect of the present invention can be stated as the development of a combination of extractants and **neutralisers** that causes neither inactivation of luciferase nor inhibition of the luciferase reaction. Only by achieving both these goals convenient and.

SUMM **Neutralisation** of an extractant can be achieved by performing a chemical reaction to destroy the extractant. The simplest example would be the **neutralisation** of an acid extractant by addition of a base. However, an exact pH adjustment would be required (strong buffers are. . . not be practicable in many situations. Furthermore the best acid extractants have chaotropic anions, which are strongly inhibitory even at **neutral** pH. Even an increased ionic strength reduces luciferase activity. An alternative approach would be to destroy the extractant by forming. . .

SUMM The most attractive approach would be to form a complex between the extracting molecule and a **neutralising** molecule. The use of nonionic **surfactants** to **neutralise** quaternary ammonium compounds (a type of cationic **surfactants**) is an example of this approach (W. J. Simpson and J. R. M. Hammond, European Patent Application 88308677.9). Actually nonionic **surfactants** **neutralise** the inactivation effect on firefly luciferase of all types of ionic **surfactants** (cationic, anionic and zwitterionic) as shown in Example 1. However, in the presence of nonionic **surfactants** all the ionic **surfactants** give an inhibitory effect at much lower concentrations than those causing inactivation. This may be due to a poor association between nonionic and ionic **surfactants** or to an inhibition from the complex between the two types of **surfactants**.

SUMM . . . to vary from sample to sample depending on the level of biological material that may bind extractants of the ionic **surfactant** type. Thus it would be necessary to use ATP standards in each assay. A further disadvantage of nonionic **surfactants** as **neutralisers** is that not all enzymes are as resistant as firefly luciferase to these agents.

SUMM The ideal compound for **neutralising** extractants would have a high association constant for the extractant. Ideally it would form an inclusion complex so that the part of the extractant molecule that inactivates enzymes is surrounded by a protective layer. Obviously the **neutralising** compound should be as inert with enzymes as possible and should not irreversibly bind **intracellular** metabolites that are of analytical interest. Some **surfactants**, e.g. the quaternary ammonium compounds, have been found to be useful extractants (A. Lundin, Extraction and automatic luminometric assay of. . . P. Stanley, G. Thorpe and T. Whitehead, Eds., pp 545-552, Academic Press, New York, 1984). A common feature of all **surfactant** molecules is a hydrophobic tail. The formation of an inclusion complex in which the hydrophobic tail is buried in a complex with a hydrophilic outer surface would be ideal. This might be achieved using a **neutralising** agent forming micelles. However, enzymes added in the analytical procedure may become incorporated into the micelles resulting in a changed activity. Furthermore an interaction between the enzymes and the extractants within the micelle can not be excluded. The ideal **neutralising** agent for **surfactants** would be a water-soluble compound with a hydrophilic outer surface not likely to bind to enzymes and a hydrophobic cave with an appropriate size to form inclusion compounds with **surfactants**.

SUMM Cyclodextrins are doughnut-shaped molecules consisting of 6, 7 or 8 glucose units (α -, β and γ - **cyclodextrin**). The internal diameter of the ring is 6 Å, 7.5 Å and 9.5 Å, respectively. The interior of the ring. . . tails of molecules as e.g. **surfactants**. The resulting inclusion complexes are generally formed with a 1:1 stoichiometry between **surfactant** and **cyclodextrin**. the association constants with α -, β and γ - **cyclodextrin** depend on the size and chemical properties of the hydrophobic tail of the **surfactant**. The association constant with **surfactants** is. . . mol.sup.-1 (I. Satake, T. Ikenoue, T. Takeshita, K. Hayakawa and T. Maeda, Conductometric and potentiometric studies of the association of α - **cyclodextrin** with ionic **surfactants** and their homologs, Bull. Chem. Soc. Jpn. 58, 2746-2750, 1985; R. Palepu and J. E. Rickardson, Binding constants of β - **cyclodextrin** /**surfactant** inclusion by conductivity measurements, Langmuir 5, 218-221, 1989; I. Satake, S. Yoshida, K. Hayakawa, T. Maeda and Y. Kusumoto. Conductometric. . . **cyclodextrins** as studied by the conductance stopped-flow method, J. Phys. Chem. 93, 3721-3723, 1989; R. Palepu and v. C. Reinsborough, **Surfactant-cyclodextrin** interactions by conductance measurements, Can. J. Chem. 66, 325-328, 1988). The outer surface of the **cyclodextrins** is hydrophilic and is. . . to this patent application **surfactants** can be removed from solutions by immobilised **cyclodextrins**. The possibility not to remove but to **neutralise** the effect of the **surfactants** by forming inclusion complexes was not evaluated. P. Khanna et al. EP 286367 describe the use of **cyclodextrins** to **neutralise** **surfactants** used as stabilisers of peptide fragments prior to assay. In a review various applications of **cyclodextrins** in diagnostics have been described (J. Szejtli, Cyclodextrins in diagnostics, Kontakte (Darmstadt) 1988 (1), 31-36). The use of **cyclodextrins** to **neutralise** **surfactants** added as extractants to release **intracellular** metabolites has not been previously described.

SUMM According to the present invention there is provided a method of preparing an extract of an **intracellular** component by providing a solution containing an **intracellular** component and a substance used for extracting the component, characterised by

contacting the solution with a **cyclodextrin** or a **cyclodextrin** derivative of an appropriate type and in an appropriate amount to **neutralise** the extracting substance. The nature of the **intracellular** component is not material to the invention. Examples are nucleic acids such as DNA and RNA and other **intracellular** metabolites as discussed above including ATP.

SUMM The term "**neutralise**" as used herein does not refer to adjustment of pH to 7.0. Rather, **neutralising** the extractants involves reducing/obviating/overcoming the interference that the extractant would otherwise cause in subsequent processing of the extracted **intracellular** component.

SUMM The function of the **cyclodextrin** or derivative is to **neutralise** the extracting substance or extractant. As discussed above, this can be done in principle by destroying the extractant. If the **cyclodextrin** or derivative is used in an insoluble form, the complex formed with the extractant is also insoluble and is readily physically removed from the remaining solution. More usually, the **cyclodextrin** or derivative is used in solution and **neutralises** the extractant by forming a complex with it. It is then possible, but usually not necessary or desirable, to remove that complex from the solution. While complete **neutralisation** of the surfactant is preferred, the invention also envisages conditions which result in partial **neutralisation**; these should significantly reduce interference by the extractant in any subsequent assay, amplification or further processing.

SUMM Appropriate corrections for dilutions and blanks (no extractant giving extracellular ATP only) were performed to assure that only **intracellular** ATP was measured. Results are shown in FIG. 11-15.

SUMM . . . E. Coli (FIG. 12) similar ATP yields were obtained with optimum concentrations of DTAB, BZC and TCA. Neither the zwitterionic **surfactant** (DDAPS) nor the anionic **surfactant** (SDS) could be used. With B. subtilis (FIG. 13), Saccharomyces cerevisiae (FIG. 14) and Chlorella vulgaris (FIG. 15) similar yields. . . be made from the data in FIGS. 11-15. Such a decision would require more elaborate experiments including e.g. studies on **cells** in different phases of growth. Furthermore if **cells** would be suspended in other media in actual samples it would be necessary to perform extractions in such media. If. . .

SUMM Use of **cyclodextrin** for **neutralising** extractants in assays of microbial ATP

SUMM . . . were tenfold diluted in Analar water. The organic material from the broth may to some extent interfere with extraction using **surfactants**. Process water may also contain some organic material but most likely at a lower level than a tenfold diluted broth..

SUMM The three extractants that according to the data in Example 2 could be used for all types of microbial **cells** (bacterial, yeast and algal) were DTAB, BZC and TCA. For each of the extractants, 10, 5 and 2.5% solutions were. . .

SUMM . . . three other process water samples (0.05 ml) extracted with an equal volume of extractant solution (0.01-3.5% DTAB). The extractant was **neutralised** by including α CD in the assay buffer (0.85 ml) to give a final concentration of 0.875% in the assay mixture. . .

SUMM Use of **Cyclodextrin** for **Neutralising** Extractants in DNA Modification or Amplification Reactions

SUMM In this experiment, the use of cyclodextrins was investigated for **detergent neutralisation** after cell

lysis. HeLa cells (10.sup.7) in 0.5 ml PBS (Sigma) were lysed by the addition of 1 ml of **Cell Lysis** Buffer (100 mM Tris, pH 8; 1 mM EDTA; 1% SDS; 0.4 mg/ml RNase A; 40 U/ml RNase T1). The. . . amounts: 10 μ l, 20 μ l, 50 μ l, 100 μ l and 200 μ l. Samples were mixed by gentle agitation prior to **analysis** of functional activity of the DNA by PCR.

SUMM . . . Only the sample with 100 μ l α CD added was digestible

with all three enzymes, indicating that these are the optimal
neutralisation conditions for both PCR and restriction
analysis of DNA in these HeLa lysates.

SUMM The PCR and restriction digest experiments indicate that α CD is
effective for SDS **neutralisation** in crude **cell**
lysates, and that DNA present in these lysates is functionally active.